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Hypothyroidism in rats decreases mitochondrial inner membrane cation permeability

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We investigated the cation permeability of liver mitochondria isolated from hypothyroid or euthyroid rats by measuring the rate of swelling of respiring mitochondria in acetate salts as a function of membrane potential. Mitochondria from hypothyroid rats have a decreased permeability of roughly 3-fold in the presence of monovalent cations K and tetramethylammonium at any (measured) membrane potential. Since the monovalent cation leak and the proton leak are known to respond similarly to membrane potential our results support the theory that the difference in non-phosphorylating respiration rate between mitochondria from hypothyroid and euthyroid rats is due to a difference in proton leak.

Thyroid hormone action; Mitochondria; Membrane potential; Proton leak; Cation leak; Respiration rate, non-phosphorylating

1. INTRODUCTION

Mitochondria isolated from hypothyroid rats characteristically show decreased respiration rates when compared with mitochondria from euthyroid controls (see [1] for a review). In a previous paper we investigated the control of non-phosphorylating respiration rate by thyroid hormone [2]. We reported that under non-phosphorylating conditions the respiration rate required to balance the same protonmotive force was less in mitochondria from hypothyroid rats than in mitochondria from euthyroid rats. This observation demonstrated that thyroid hormone controls the rate of non-phosphorylating respiration by one of the two following possible mechanisms: (i) thyroid hormone controls the proton leak across the mitochondrial inner membrane such that at any given protonmotive force mitochondria from hypothyroid rats have a smaller proton leak than mitochondria from euthyroid controls; (ii) thyroid hormone controls the mitochondrial H^+/O ratio such that at any given protonmotive force mitochondria from hypothyroid rats have a higher H^+/O ratio than mitochondria from euthyroid rats.

We have previously shown that at low (unmeasured) values of protonmotive force mitochondria from hypothyroid and euthyroid rats have the same H^+/O ratio [3]. Possibility (ii) therefore depends on the mitochondrial H^+/O ratio decreasing at high values of protonmotive force, and thyroid hormone controlling the dependence of this decrease on protonmotive force. Whether the mitochondrial H^+/O ratio is not fixed but decreases at high values of protonmotive force is currently a matter of debate, largely as a result of the difficulties involved in measuring mitochondrial proton permeability unambiguously (cf. [4–8]).

Brown and Brand [6] have introduced a method that allows measurement of the membrane potential dependence of monovalent cation uptake into

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Abbreviations: H^+/O, number of protons released to the external bulk phase/oxygen atom reduced; Ph_3MeP^+, methyltriphenylphosphonium cation

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isolated mitochondria. They observed that the monovalent cation leak and the endogenous proton leak appear to depend in a similar manner on the membrane potential. In the present paper we test for a change in proton conductance in different thyroid states by measuring the relative mitochondrial cation permeabilities, thus avoiding the possible ambiguities that arise in measuring proton permeability directly. We show that at high values of protonmotive force mitochondria from hypothyroid rats experience a roughly 3-fold decrease in permeability due to K^+ and tetramethylammonium$^+$ in comparison with mitochondria from euthyroid rats. This supports the hypothesis that a (non-specific) change in leak across the mitochondrial inner membrane underlies the change in mitochondrial non-phosphorylating respiration rates under different thyroid states.

2. MATERIALS AND METHODS

Preparation of hypothyroid rats (by parathyroidthyroidectomy) and mitochondria were as described in [2]. Measurement of mitochondrial volume from the distribution of ^3H_2O and [^{14}C]sucrose, mitochondrial membrane potential from the accumulation of [^{3}H]Ph_3MeP^+ and Ph_3MeP^+-binding correction were as described in [9]. The same Ph_3MeP^+-binding correction (0.36) was used for mitochondria from hypothyroid and euthyroid rats. This may have caused a slight underestimate of membrane potential for mitochondria from hypothyroid rats (cf. [2]).

Measurement of mitochondrial inner membrane cation permeability was performed as in [6]. This involves measuring the rate of swelling of respiring mitochondria in the acetate salt of a cation at different membrane potentials (set by titrating with the respiratory chain inhibitor malonate). Beavis et al. [10] and Garlid and Beavis [11] have shown that the reciprocal absorbance of mitochondrial suspensions can be used as a quantitative measure of mitochondrial volume (and thus rate of solute uptake). Selwyn [12] has shown that transmittance may be used instead of reciprocal absorbance without introducing much error when relative rates of solute transport are required (as here). The rate of mitochondrial swelling (and thus relative rate of solute transport) was therefore followed from the change in %transmittance at 540 nm, in a 3 ml cuvette continuously stirred by an air driven paddle in a Perkin Elmer Lambda 5 spectrophotometer. Initial values of %transmittance were approximately 1%. Measurements of swelling rate were made in the range 1–3%transmittance. This is slightly outside the optimal range; as a consequence %transmittance represents an overestimate of approximately 15% of the true rate of cation entry at low membrane potential when compared to the values at high membrane potential. Measurement of tetramethylammonium$^+$ permeability was as follows: mitochondria (3 mg of protein) were incubated in 3 ml of 120 mM tetramethylammonium acetate, 4 mM succinate, 1 mM EGTA, 5 μM rotenone, 5 μM Ph_3MeP^+, 1 μg oligomycin/ml, pH 7.2 (with tetramethylammonium hydroxide), at 37°C, in the presence of 0, 0.8, 1.6 or 2.4 mM tetramethylammonium malonate. The rate of %transmittance increase was followed at 540 nm. This was roughly linear between 0.5 and 2.5 min. Membrane potential was measured in parallel incubations from the distribution of [^{3}H]Ph_3MeP^+; mitochondria were incubated for 2 min in incubation medium supplemented with [^{3}H]Ph_3MeP^+ before centrifugation for 2 min. Measurement of K^+ permeability followed the same protocol, with K^+ replacing tetramethylammonium$^+$ in all instances. All chemicals were obtained from the sources indicated in [2]. Experimental design was as described in [2].

3. RESULTS AND DISCUSSION

Fig.1 shows the permeability to tetramethylammonium$^+$ (measured as rate of change of %transmittance) of mitochondria from hypothyroid and euthyroid rats plotted against membrane potential. Fig.2 shows the permeability to K^+ (measured as rate of change of %transmittance) of mitochondria from hypothyroid and euthyroid rats plotted against membrane potential. Control experiments showed that equivalent changes in mitochondrial volume produced equivalent changes in %transmittance for mitochondria from hypothyroid and euthyroid rats. Thus the same change in %transmittance represents the same volume change and therefore the same rate of cation entry for mitochondria from hypothyroid and euthyroid rats. Our determination of mitochonrial membrane potential involves a 2 min centrifugation. We have observed that pelleting of the mitochondria is complete within 15–30 s. The observed rate of change of %transmittance was linear between 30 s and 2.5 min; thus the plots of change in %transmittance against membrane potential are comparable even though we do not know exactly when pelleting occurred. The figures therefore show that at any given membrane potential the rate of cation entry is approximately 3-fold less in mitochondria from hypothyroid rats compared to mitochondria from euthyroid rats. Note we have not considered the contribution of the transmembrane pH gradient in these plots as it is not a driving force for monovalent cation accumulation.

The permeabilities of the mitochondrial inner membrane to monovalent cations and to protons respond in a similar manner to membrane poten-

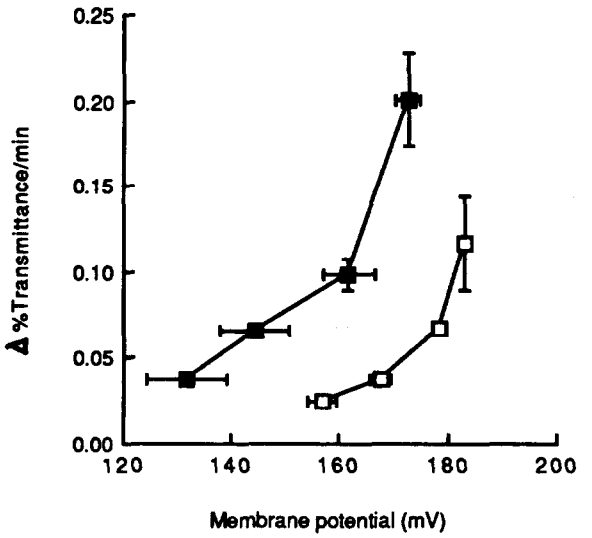

Fig.1. Rate of swelling in tetramethylammonium acetate versus membrane potential for mitochondria from hypothyroid (□) and euthyroid rats (■). Data are from 3 independent experiments; error bars represent standard error of the mean.

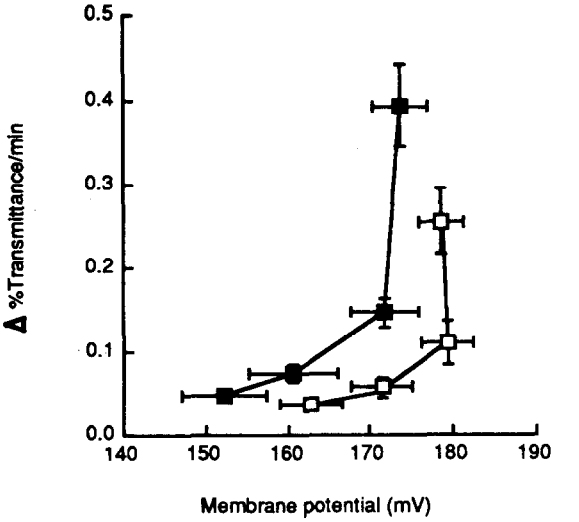

Fig.2. Rate of swelling in potassium acetate versus membrane potential for mitochondria from hypothyroid (□) and euthyroid rats (■). Data are from 3 independent experiments; error bars represent standard error of the mean.

tial [6]. We can therefore take the 3-fold decrease in relative cation permeability of mitochondria from hypothyroid rats (compared to mitochondria from euthyroid rats) as a strong indication of a 3-fold decrease in proton permeability. Our earlier investigation into the control of non-phosphorylating respiration rate by thyroid hormone showed that at any given protonmotive force the rate of respiration was approximately 3-fold less in mitochondria from hypothyroid rats compared to mitochondria from euthyroid rats [2]. This observation can be entirely explained by a 3-fold decrease in proton permeability in mitochondria from hypothyroid rats, without needing to invoke changes in the mitochondrial H^+/O ratio. The data presented here are therefore consistent with the hypothesis that thyroid hormone controls the rate of non-phosphorylating respiration by controlling the proton leak across the mitochondrial inner membrane. This conclusion is strengthened by recent observations and arguments [8] that weaken the case for changes in the mitochondrial H^+/O ratio at high protonmotive force.

The non-specific nature of the decrease in the cation permeability of mitochondria from hypothyroid rats lends support to the theory postulated in [2] that the change in proton conductance in different thyroid states results from changes in the mitochondrial inner membrane surface area and/or fatty acid composition rather than changes in the activity of a proton conducting channel of the type found in brown fat [13].

The conclusion that thyroid hormone controls the rate of non-phosphorylating mitochondrial respiration by changing the proton leak may appear similar to the older hypothesis that the action of thyroid hormone is to uncouple mitochondrial respiration (see [14] for a review). Triiodothyronine and thyroxine presumably are capable of acting as uncouplers (at unphysiological concentrations) through a protonation/deprotonation of the terminal hydroxyl group in a manner analogous to the uncoupler dinitrophenol. Such a mechanism would not be expected to carry K^+ or tetramethylammonium$^+$, arguing against such an uncoupling process being responsible for the altered proton conductance of mitochondria from hypothyroid rats.

We conclude that mitochondria isolated from hypothyroid rats have a 3-fold decreased cation conductance compared to mitochondria from euthyroid rats. This probably reflects a 3-fold decreased proton conductance that explains the altered relationship between protonmotive force and respiration rate as well as the decreased non-

phosphorylating respiration rate of mitochondria from hypothyroid rats compared to mitochondria from euthyroid rats.

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